Australian Government
Department of Defence
Defence Science and
Technology Organisation

Treatment of Biofouling in Internal Seawater Systems - Phase 2

John A. Lewis and Jim Dimas

Maritime Platforms Division
Defence Science and Technology Organisation

DSTO-TR-2081

ABSTRACT

Biofouling in the internal seawater systems of vessels is considered to pose a high risk for the introduction and/or translocation of marine pests and, as part of Australia's new National System for the Prevention and Management of Marine Pest Incursions, options for treating such biofouling are needed. Mussels are of particular concern. In this study, a range of chemicals, including vinegar, detergents, disinfectants, bleach, descalers, pipework treatments and freshwater, were tested on the southern Australian blue mussel, *Mytilus galloprovincialis planulatus*. The effectiveness of descalers in digesting mussel shells was assessed, and the toxicity of other treatments determined in 6 and 14 h (hour) exposures. The most effective treatments were two disinfectants, which both contained the active benzalkonium chloride. 14 h treatments with disinfectants of this type were concluded to be the most effective means of killing mussels. However, the toxicity and environmental acceptability of these chemicals warrant investigation in regard to discharge and disposal of treatment solutions.

RELEASE LIMITATION

Approved for public release

Published by

Maritime Platforms Division
DSTO Defence Science and Technology Organisation
506 Lorimer St
Fishermans Bend, Victoria 3207 Australia

Telephone: (03) 9626 7000
Fax: (03) 9626 7999

AR-014-067
November 2007

APPROVED FOR PUBLIC RELEASE

Treatment of Biofouling in Internal Seawater Systems - Phase 2

Executive Summary

Australia is developing a National System for the Prevention and Management of Marine Pest Incursions that includes best practice management guidelines to address marine biofouling risks. Biofouling in internal seawater systems is one niche area on vessels that is considered to pose a high risk for the introduction and/or translocation of marine pests. Options for treating biofouling in internal seawater systems will therefore be included in industry sector guidelines. The aim of this project, undertaken for the Invasive Marine Species Program within the Australian Government Department of Agriculture, Fisheries and Forestry, is to assess the efficacy of potential treatment chemicals for this application.

In 1999, following the incursion of the black-striped mussel into Darwin marinas, the Northern Territory government adopted a protocol requiring the dosing of pipework on incoming vessels with a 5% detergent solution, based on experimental work at the University of the Northern Territory. However, concerns have since been raised about both the efficacy of detergents in killing mussels, and the environmental acceptability of the practice. Subsequently, a study by CRC Reef compared the efficacy of a disinfectant and vinegar against the Sydney rock oyster and found vinegar to be the more effective, but without achieving 100% effectiveness.

In this study vinegar and a range of other chemicals, including detergents, disinfectants, bleach, descalers, commercial pipework treatments and freshwater, were tested on the southern Australian blue mussel *Mytilus galloprovincialis planulatus.* The effectiveness of descalers in dissolving mussel shells was assessed, and the toxicity of other treatments determined after 6 and 14 h exposures. Of the latter, all treatments except freshwater caused some mortality. However, the most effective treatments were the disinfectants *Conquest* and *Quatsan*, which both contain the active benzalkonium chloride. These two treatments caused 100% mortality within 48 h of immersion for 14 h at concentrations of 1% and above. Other treatments were less effective. The descaler *Rydlyme* did fully digest mussel shells at a concentration of 25% but use of this treatment is not considered practical because of the linear relationship between acid volume needed and total mussel abundance and biomass.

14 h treatments with disinfectants of the type tested are concluded to be the most effective means of killing mussels, or at least for the test organism used in this study. However, the toxicity of these chemicals does warrant further investigation in relation to the environmental acceptability and means of discharge and disposal of the treatment solution. Approval by the Australian Pesticides and Veterinary Medicines Authority (APVMA) may also be required for use in a biofouling control application.

Authors

John A. Lewis
Maritime Platforms Division

Since 1977, after completing BSc (Hons) and MSc degrees in marine biology at the University of Melbourne, John Lewis has worked as a scientist in the Defence Science & Technology Organisation, with primary research interests in marine biofouling and its prevention, and the effects of RAN activities on the marine environment. John currently heads the Environmental Compliance and Biotechnology Group, within the DSTO Maritime Platforms Division, and leads a team investigating new, environmentally acceptable methods of biofouling control, biofouling and marine pest management, environmental compliance of naval vessels, and other environmental aspects of navy operations.

Jim Dimas
Maritime Platforms Division

Jim started working in DSTO in 1987 as a trainee technical officer. He completed his traineeship in 1990 after gaining his AsscDip (Laboratory Technology) at the Northern Melbourne Institute of TAFE. In the same year he started working in the Ballistic Protection and Survivability area within the then Materials Division. In 1998 he gained his BSc (Applied Chemistry) at RMIT University. In 2000 he transferred to the Environmental Compliance and Biotechnology Group within the Maritime Platforms Division. In 2002 he completed his Certificate of Surface Coatings, (Surface Coatings Association Australia Inc SCAA) at Victoria University. Currently he is working on novel antifouling strategies.

Contents

1. Introduction

Australia is developing a National System for the Prevention and Management of Marine Pest Incursions (the National System) and, within the National System, best practice management guidelines are being developed that will address marine pest biofouling risks. Biofouling of vessel niche areas, including internal seawater systems, sea chests, anchors, bilges and equipment have been identified as high risk areas for the introduction and/or translocation of marine pests. Options for treating internal seawater systems, to kill any organisms that may have colonised there, will be included in industry sector biofouling management guidelines.

The aim of this project was to assess the efficacy of a number of potential treatment chemicals for removing biofouling from internal seawater systems on marine craft. The project was undertaken for the Invasive Marine Species Program (IMSP) in the Corporate Policy Division within the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF) with funding sourced from the National Heritage Trust (NHT).

2. Background

The prevention of new incursions of invasive marine pests into Australia requires a procedure to treat the internal seawater systems of vessels to kill any potential pests that may have colonised those systems.

Particular species of concern include the black-striped mussel, *Mytilopsis sallei* (BSM), and the Asian green mussel, *Perna viridis* (AGM). Following the BSM incursion into Darwin marinas in 1999, the Northern Territory adopted a protocol requiring the dosing of pipework on incoming vessels with a 5% detergent solution and holding this solution in the system for a minimum period of 14 h. The protocol was based on studies completed by the University of the Northern Territory at the time of the BSM incursion that found several detergents effective in killing this species (D. Parry, pers. comm.)

However, concerns have been raised about both the efficacy of detergents in killing mussels, and environmental issues associated with the discharge of treated water into marinas and other inshore waters. As a consequence, a project was undertaken for IMSP by CRC Reef to investigate the efficacy of a detergent/disinfectant and a number of alternative treatments against the Sydney rock oyster *Saccostrea glomerata* (Neil and Stafford, 2005). This study compared the efficacy of varied concentrations of two chemical products: *Quatsan* ® (active: benzalkonium chloride) and vinegar (active: acetic acid). This study concluded that a 10% vinegar concentration was most effective over a 12 h period, yielding 75% average mortality.

The aim of the present project was to assess the efficacy of vinegar against the southern Australian blue mussel *Mytilus galloprovincialis planulatus* and, at the same time, to screen a number of other potential treatment chemicals. These additional chemicals included detergents and disinfectants, bleach, because of the known biocidal activity of hypochlorite, and several descalers and pipework treatment chemicals. Descaling solutions, often strong

acids or alkalis, are industrial chemicals used to dissolve calcareous deposits ("scale") and corrosion products in cooling water systems to remove blockages and improve the performance of heat exchangers. Pipework treatment chemicals are products which, with regular use, are claimed to keep pipework free of marine growth and organic, scale and similar deposits.

3. Materials & Methods

3.1 Treatment Chemicals

The following categories of chemicals were included in the study: 1. Vinegar; 2. Detergent; 3. Disinfectant; 4. Bleach; 5. Pipework treatments; 6. Freshwater; and 7. Descalers

The products tested were as follows.

1. Vinegar:
   - Co*rnwell's White Vinegar* (Meadow Lea Foods, Mascot)
     Chemical composition[1]:

Acetic acid	4%

2. Detergents:
   - *Dobatex Gold* ® (ex *Teepol*) (Shell Company of Australia, Melbourne)
     Chemical composition[2]:

Triethanolamine	<5%
Alkylbenzene sulphonic acid	<5%

   - *Palmolive Original* (Colgate-Palmolive, Sydney)
     Chemical composition[3]:

Sodium dodecyl benzene sulfonate	30-50%
Formaldehyde	0-0.1%
Benzyl salicylate	0-0.1%
Octanal, 2-(phenylmethylene)	0-0.1%

3. Disinfectants:
   - *Conquest* ® (Shamrock Chemicals (N.T.) Pty Ltd, Winnellie, NT)
     Chemical composition[4]:

Quaternary ammonium compounds	<10%
Surfactants	<10%
Sequestrants	<10%

[1] www.gffoodservice.com.au. Accessed: 22/09/2006
[2] Safety Data Sheet: Shell Dobatex Gold. Version No 1.1. 27/04/2004 www.shell.com
[3] Material Safety Data Sheet: Palmolive Dishwashing Liquid. Issue Date: October 2003. Colgate
[4] Material Safety Data Sheet: Conquest. Issue Date: February 2004. Shamrock Chemicals (N.T.) Pty Ltd

Dipropyleneglycol methyl ether	<10%
Alkaline salts	<10%

- *Quatsan* ® (Northern Chemicals Pty Ltd, Cairns)
  Chemical composition[5]:

Quaternary ammonium compound	10-60%
Benzalkonium chloride	<10%
Non-ionic surfactants	<10%
Alkaline salts	<10%

4. Bleach:
   - *White King* ® *Regular* (Sarah Lee, Clayton South)
     Chemical composition[6]:

Sodium hypochlorite	1-10%
Sodium hydroxide	0-1%

5. Pipework treatments:
   - *SWT Ecosperse* ® *Liquid sea water cooling system antifoulant and corrosion inhibitor* (Port Marine Pty Ltd, Artarmon)
     Chemical composition[7]:

Neutralised alkylamine	15%
Biocide	<5%
Surfactants	Unspecified

   - *Triple 7 Colloidal Concentrate* (Triple 7, Warrnambool)
     Chemical composition[8]:

Tall oil fatty acids	<5%
Alcohol ethoxylate	5%
Aspartic acid	<1%
N-(1,2-dicarboxyethyl)-tetrasodium salt	<1%

6. Descalers:
   - *Rydlyme* (Rydlyme, WA)
     Chemical composition[9]:

Hydrogen chloride, aqueous	< 10%

   - *Triple 7 Enviroscale* (Triple 7, Warrnambool)
     Chemical composition[10]:

[5] Material Safety Data Sheet: Quatsan. Issue Date: 27/01/2004. Northern Chemicals Pty Ltd
[6] Material Safety Data Sheet: White King™ Premium Bleach. Issue Date: June 2004. Sarah Lee
[7] Material Safety Data Sheet: SWT Ecosperse. Issue Date: 18 March 2003. Uniservice International
[8] Material Safety Data Sheet: Triple 7 Colloidal Concentrate. Issue Date: 14 Dec 2005. Triple 7 Environmental Chemistries
[9] Material Safety Data Sheet: Rydlyme. Issue Date: Aug 2000. APEX Engineering Products Corp., Plainfield, IL

Lactic acid	< 2%
Citric acid	< 2%
Non-ionic surfactant	Not specified

- *Steradent Extra Strength Denture Cleansing Tablets* (Reckit Benckiser, West Ryde)
  Chemical composition[11]:

Potassium peroxymonosulfate	10-30%
Sodium carbonate	<10%
Citric acid	30-60%
Sodium sulfate	10-30%
Sulfamic acid	<10% (40g/kg)
Malic acid	<10%
Sodium dodecyl benzene sulfonate	<10%

3.2 Test Organisms

Australian blue mussels, *Mytilus galloprovincialis planulatus*, were collected from the lower intertidal zone on wooden structural supports of Booth Pier in Hobsons Bay (northern Port Phillip Bay), Victoria. Booth Pier is within the Tenix Williamstown Shipbuilding Facility, and is the location of the DSTO Marine Coatings and Corrosion Test Facility, with the marine test raft that forms part of this facility moored against Booth Pier. The annual average seawater temperature range at 1 m depth at this location is 10.5 – 21.5°C (DSTO, unpublished data).

Mussels were collected under a permit (RP857) issued under the Fisheries Act 1995 by the State Government of Victoria Department of Primary Industries.

Batches of mussels, sufficient to allow two weeks of testing, were transferred to the DSTO laboratories in Maribyrnong in a tub of seawater. In the laboratory, individual mussels were separated by cutting the byssal threads, and transferred to a 54 l aerated, filtered aquarium containing natural seawater at ambient temperature (~16°C)[12]. Experiments were performed between September and December 2006, at which time seawater temperatures at the field collection site increased from approximately 12 to 19°C. The average length of mussels used for testing was around 45 mm (range 25 – 65 mm).

[10] ChemWatch Material Safety Data Sheet: Triple 7 Enviroscale (Acid). Issue Date: Jul 2004.
[11] Product Safety Data Sheet: Steradent Extra Strength Denture Cleansing Tablets. Issue Date: Feb 2006. Reckitt Benckiser
[12] In the first instance, in July 2006, mussels were transferred directly to an aquarium in the 20°C constant temperature room. All mussels died within 24 h, apparently due to temperature shock (surface seawater temperatures at Booth Pier at this time were < 11°C). The procedure was subsequently modified to pass collected mussels through an equilibration tank in an unheated laboratory and no further losses were experienced.

3.3 Test Method

Test dishes were prepared by gluing squares of black plastic mesh (cut from mussel spat settlement "Christmas tree rope" (Netcraft Pty Ltd, Margate, Tasmania)) inside disposable plastic petri dishes. For all chemical tests except for the descalers, at least 24 h before the conduct of a test series, five individual mussels were placed in each test dish and the test dishes transferred to an aerated, filtered aquarium holding tank of natural seawater in a 20°C constant temperature room. Byssal reattachment was used as an indicator that test mussels were alive before testing.

Test exposures were performed in 5 l glass beakers containing 3 l of artificial seawater (*Instant Ocean* ®, Aquarium Systems Inc., Mentor, OH) aerated by bubbling air through a pasteur pipette. One test dish was placed in each beaker and the treatment chemical added to achieve the required test concentration. Freshwater test solutions were prepared using artificial seawater prepared and diluted with de-ionised water. The types and concentrations of chemicals tested are listed in Table 1. For any one chemical, a series of test concentrations was assessed concurrently along with a control with no added test chemical. A replication of all test series was undertaken after the first round of testing.

Tests were undertaken for 6 and 14 h. After the exposure period, test dishes were removed from the test beakers, the byssal threads again cut, and the test dishes transferred to an aerated, filtered aquarium recovery tank, also in the constant temperature room. Mortality of mussels was assessed when removed from the test beaker, and at 24 and 48 h after completion of the test. Mortality was recorded when mussels floated or remained open when removed from water.

For the descalers, as the active process is dissolution of calcareous deposits, these, except for *Steradent*, were assessed by immersing single mussels in 250 ml beakers containing 150 ml of 0, 1, 5, 10, 25 and 50% v/v treatment chemical in artificial seawater. Hydrochloric acid (35%, analytical grade, Biolab (Aust) Ltd) was tested alongside the commercial descalers. *Steradent* was tested by using multiple tablets. Mussels were weighed before and after 24 h immersion.

Table 1. Treatments and treatment concentrations

Test No.	Treatment Category	Chemical	Treatment Concentration				
			1	2	3	4	5
1a	1	Vinegar	Control (0%)	5% v/v	10% v/v	25% v/v	50% v/v
1b		As above					
2a	2	Detergent 1 *Dobatex*	Control (0%)	1% v/v	5% v/v	10% v/v	
2b		As above					
3a	2	Detergent 2 *Palmolive*	Control (0%)	1% v/v	5% v/v	10% v/v	
3b		As above					
4a	3	Disinfectant 1 *Conquest*	Control (0%)	1% v/v	5% v/v	10% v/v	
4b		As above					
5a	3	Disinfectant 2 *Quatsan*	Control (0%)	1% v/v	5% v/v	10% v/v	
5b		As above					
6a	4	Bleach *White King*	Control (0%)	1% v/v	5% v/v	10% v/v	
6b		As above					
7a	6	Pipework Treatment 1 *Colloidal Concentrate*	Control (0%)	1% v/v	5% v/v	10% v/v	
7b		As above					
8a	6	Pipework Treatment 2 *SWT Ecosperse*	Control (0%)	1% v/v	5% v/v	10% v/v	
8b		As above					
9a	7	Freshwater	Control (33 ppt)	10 ppt salinity	5 ppt salinity	0 ppt salinity	
9b		As above					

4. Results

4.1 Vinegar

Mortality of mussels in both the 6 and 14 h immersions in vinegar were variable (Figures 1 & 2). For 6 h immersions, in all test concentrations some mussels died during the immersion period. 48 h after removal from the vinegar solution, all mussels were dead in both 10% and 50% treatments and in one of the 25% tests. However, in the 14 h tests, some mussels survived in some tests at all test concentrations. No mortality occurred in the controls.

An observation made during these tests was that, in the 50% treatments, the shells of barnacles (*Austrominius* spp.) growing on the mussel shells were completely dissolved. However, there was no apparent dissolution of the mussel shells, although vinegar concentrations of 25 and 50% degraded the adhesion of the periostracum to the underlying shell and this partially lifted and peeled.

4.2 Detergents

For detergent 1, *Dobatex*, only a few mussels died after 6 h exposures (Figure 3). Mortality was higher after 14 h immersion (Figure 4) but, at all concentrations, there was no mortality in at least one test.

Detergent 2, *Palmolive* dishwashing liquid, had a greater effect and some mussels were killed after both 6 and 14 h immersions at all test concentrations (Figures 5 & 6). In the 6 h tests, results suggest a direct relationship between test concentration and mortality, with mortality highest at 10%, and lowest at 1%, but there were some survivors after 48 h from all tests. Mortality was higher in 14 h tests, and all mussels died in some tests. However, this was not consistent with concentration and some mussels survived in all concentrations in one of the tests.

In both of the detergent tests, there was a large amount of foam generated, with foam overflowing from the test beakers.

4.3 Disinfectants

The highest mortality observed for disinfectant 1, *Conquest*, after 6 h immersion was at the lowest concentration, 1% v/v (Figure 7). In both tests, a 1% concentration killed all mussels, but in one test at each of 5% and 10% there were survivors. Perhaps unexpectedly, the effect of a 1% solution was greatest, 5% the least, and 10% in between.

After 14 h immersion in the test solution, all mussels from all test concentrations were dead within 48 h of removal from the test solutions (Figure 8).

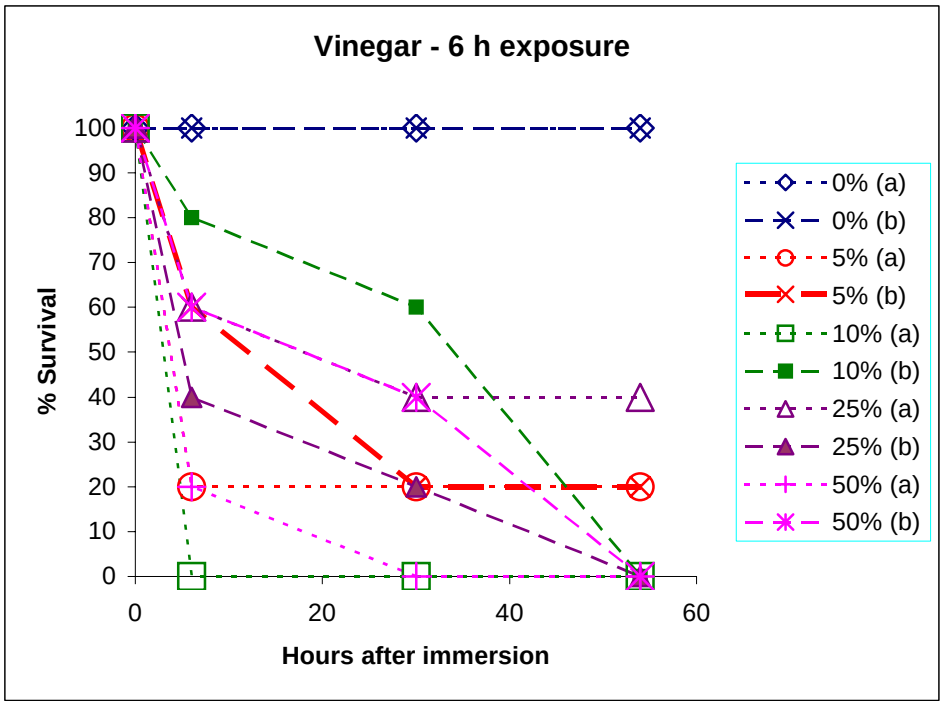

Figure 1. Test results for vinegar (6 h exposure)

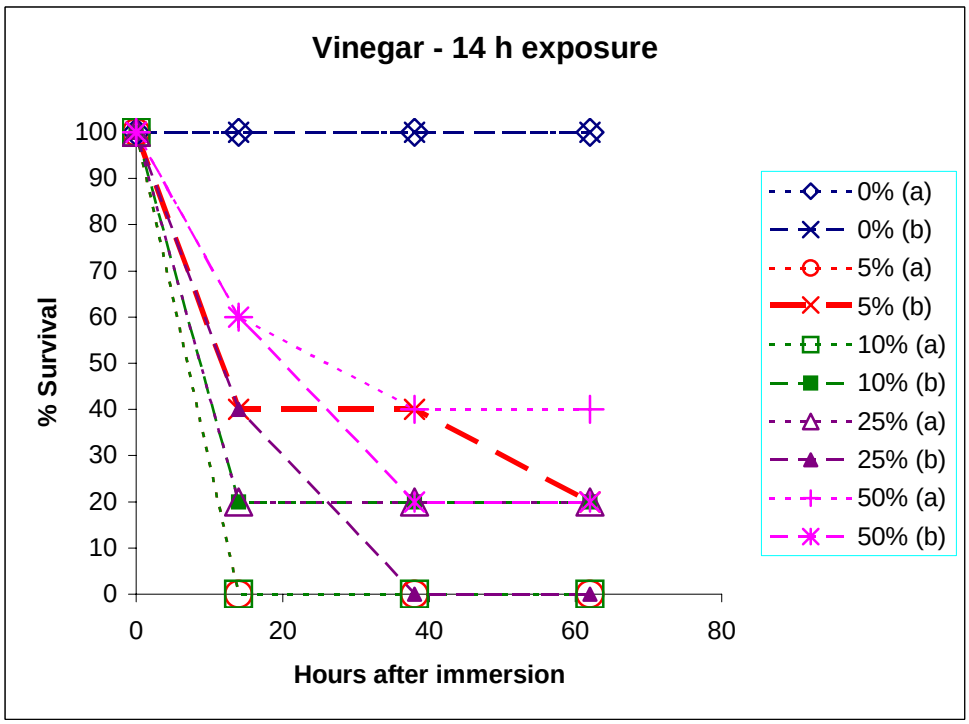

Figure 2. Test results for vinegar (14 h exposure)

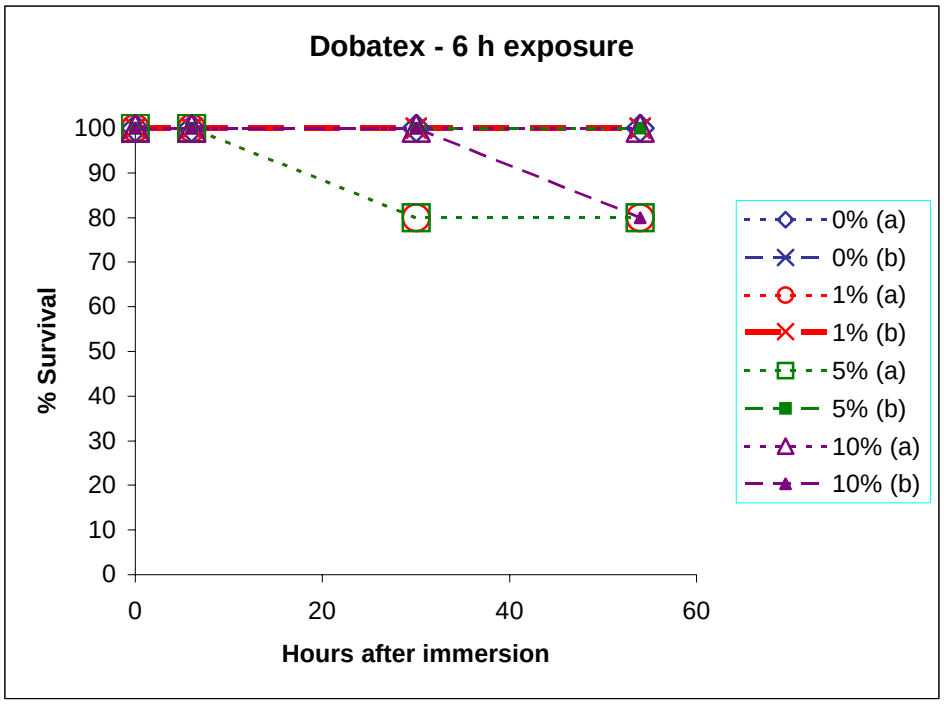

Figure 3. Test results for Detergent 1 (6 h exposure)

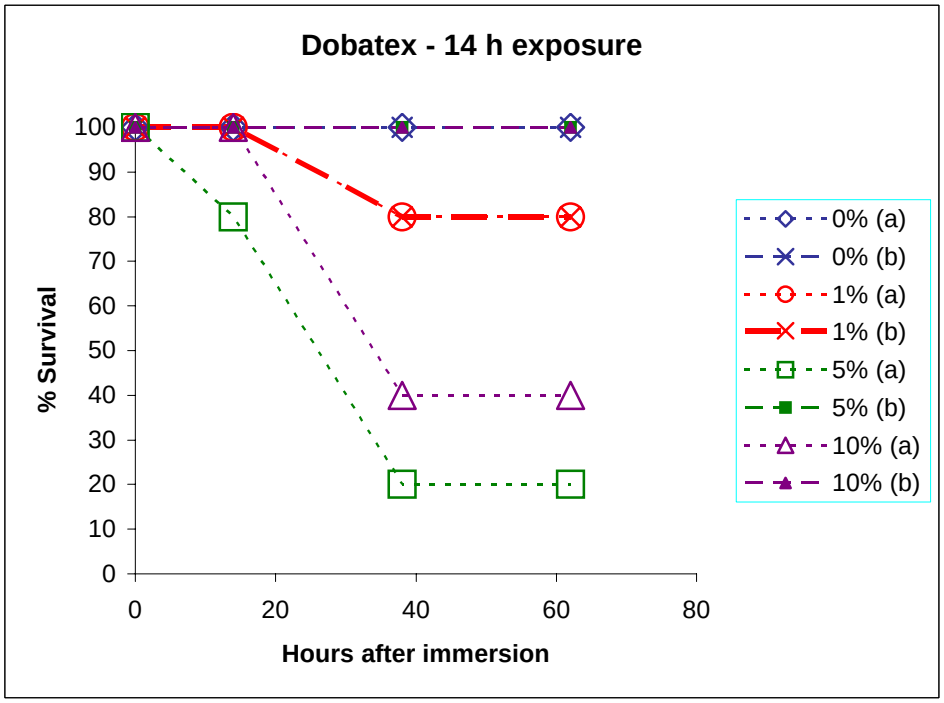

Figure 4. Test results for Detergent 1 (14 h exposure)

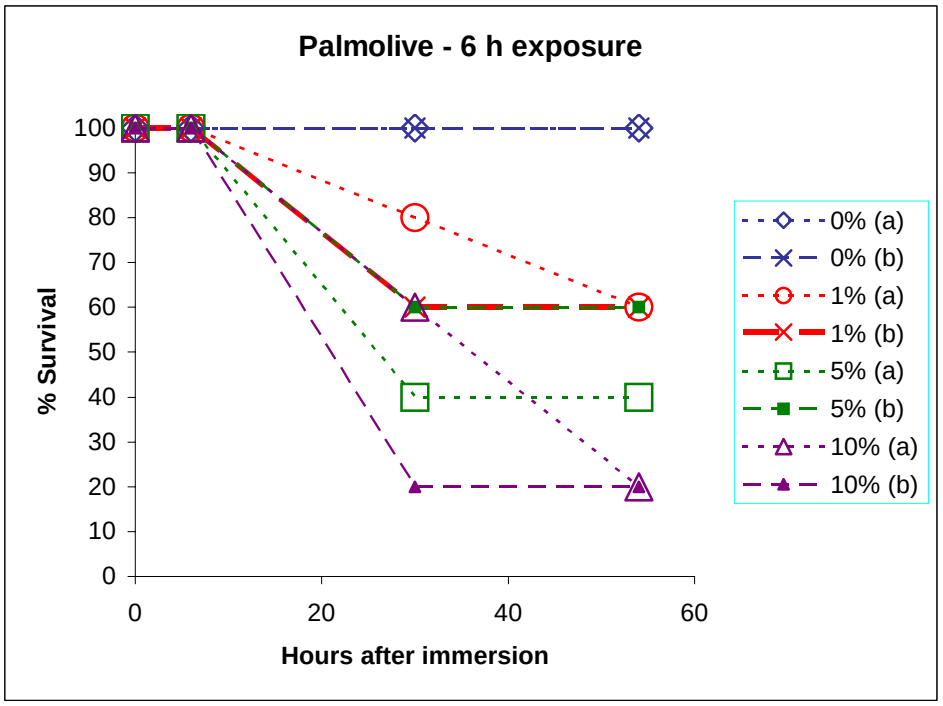

Figure 5. Test results for Detergent 2 (6 h exposure)

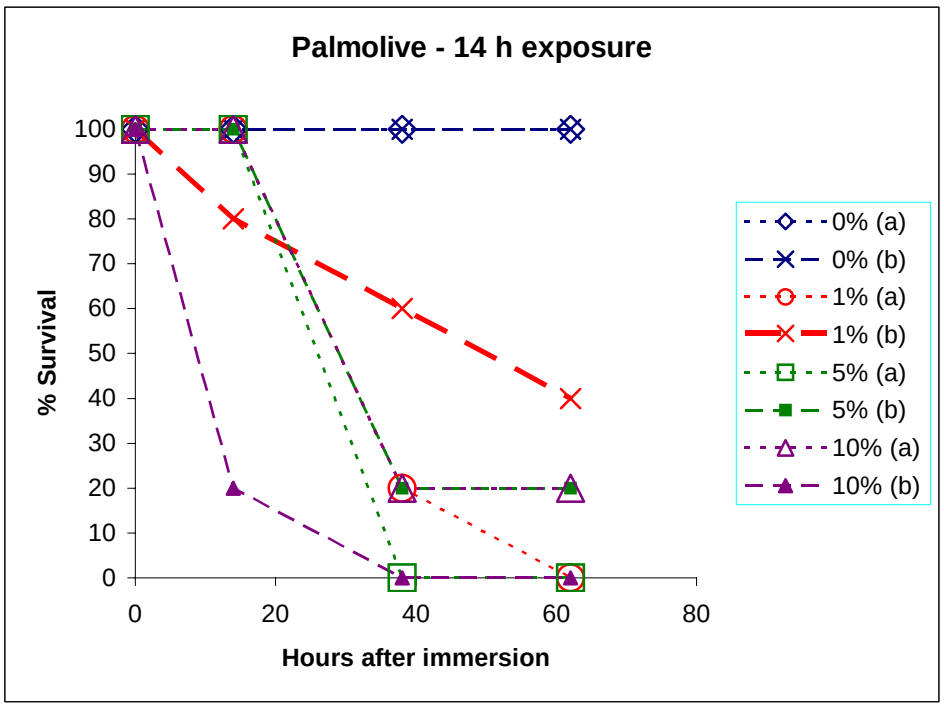

Figure 6. Test results for Detergent 2 (14 h exposure)

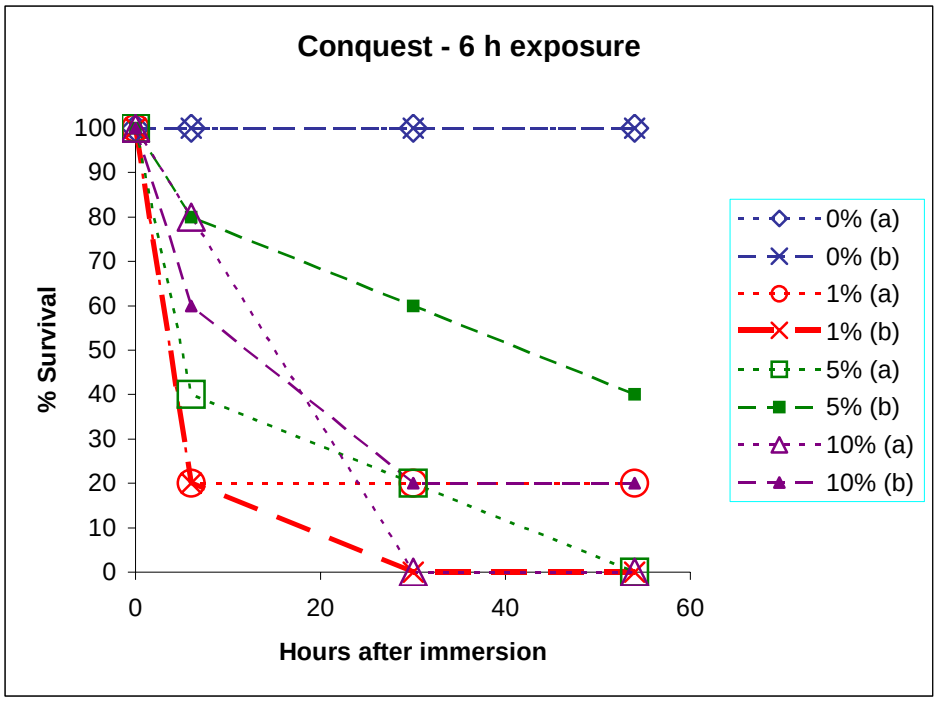

Figure 7. Test results for Disinfectant 1 (6 h exposure)

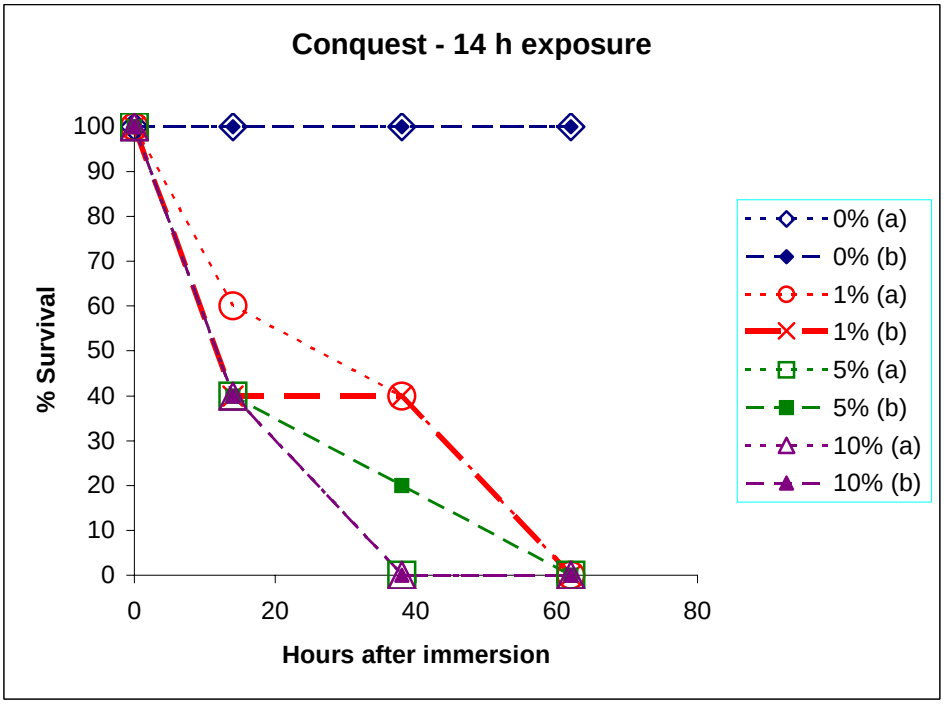

Figure 8. Test results for Disinfectant 1 (14 h exposure)

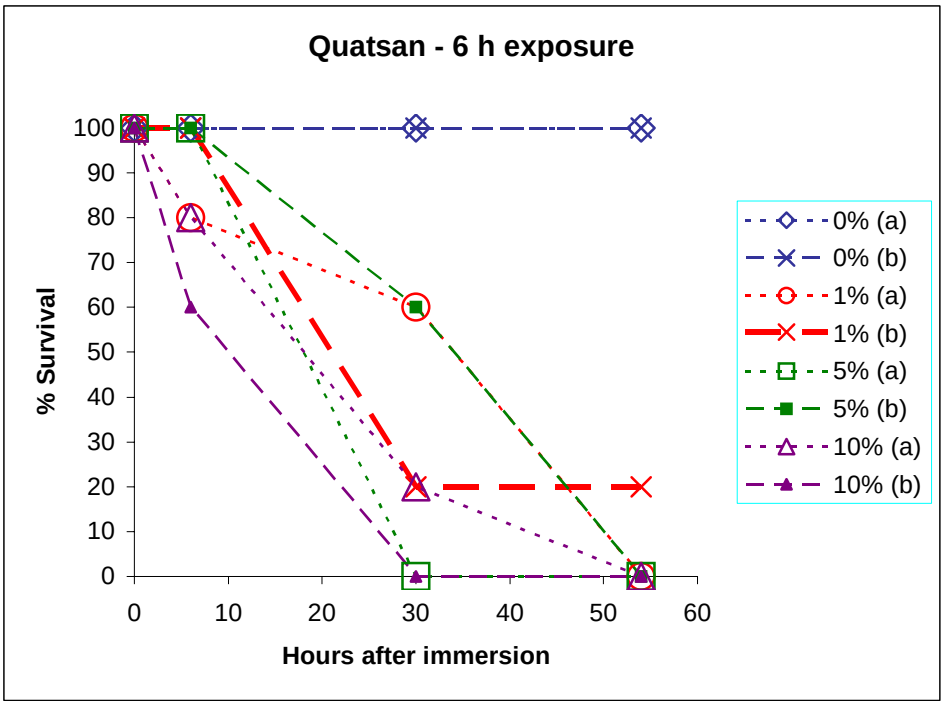

Figure 9. Test results for Disinfectant 2 (6 h exposure)

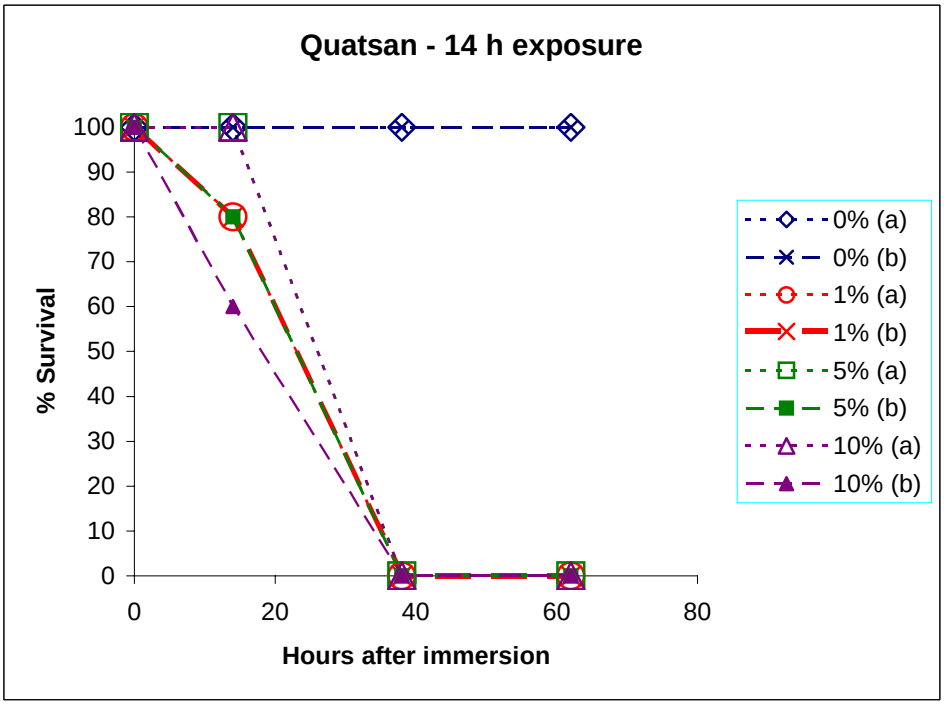

Figure 10. Test results for Disinfectant 2 (14 h exposure)

Results were similar for disinfectant 2, *Quatsan*, but higher mortality occurred after 6 h than occurred in the *Conquest*. Mussel survival was only seen in one test of 1% (Figure 9). For 14 h tests, all mussels in all test concentrations were confirmed dead within 24 h of removal from the test solutions (Figure 10).

As with the detergents, disinfectant treatments foamed significantly.

4.4 Bleach

The bleach, *White King* ®, caused mortality in all tests at concentrations greater than 1% for 6 and 14 h, and the effect tended to increase with concentration (Figures 11 & 12). However, in all tests some mussels did survive. An effect noted for the bleach was that, at treatment concentrations greater than 5%, the byssal threads were dissolved and mussels detached. Mussels did survive this and produced new byssal threads after transfer to the recovery tank.

4.5 Pipework Treatments

Some mussels immersed at all test concentrations of *Triple 7 Colloidal Concentrate* for 6 h died (Figure 13). Full mortality only occurred in one test at a concentration of 10%. Mortality was higher after 14 h immersion (Figure 14). The 10% concentration killed all mussels in both tests, but some mussels survived in one test at both 1% and 5% concentrations.

After 6 h immersion in *SWT Ecosperse* all mussels had died within 48 h of removal at all test concentrations (Figure 15). Results were similar for the 14 h tests, except for one test at 5% concentration in which there were some survivors (Figure 16).

4.6 Freshwater

No mortality of mussels occurred from 6 or 14 h immersions in reduced salinity water, including the zero salinity water (Figures 17 & 18).

4.7 Descalers

Rydlyme, *Enviroscale* and hydrochloric acid all at least partially digested the mussel shells, but *Steradent* had little effect (Figure 19). Hydrochloric acid digested approximately 50% of the mussel mass at concentrations > 5%, and *Rydlyme* had a similar effect at > 25%.

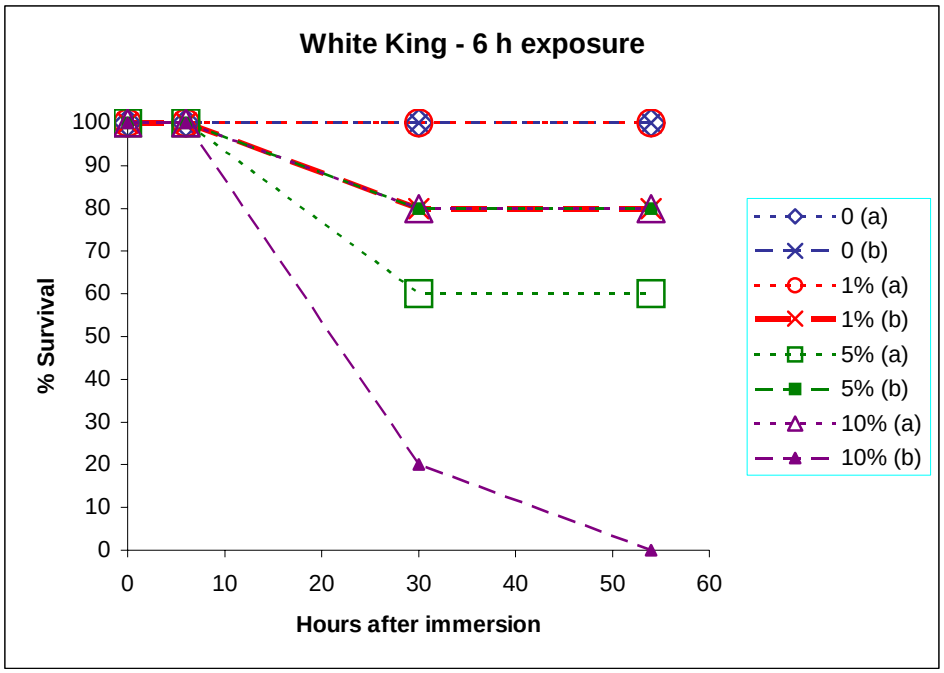

Figure 11. Test results for bleach (6 h exposure)

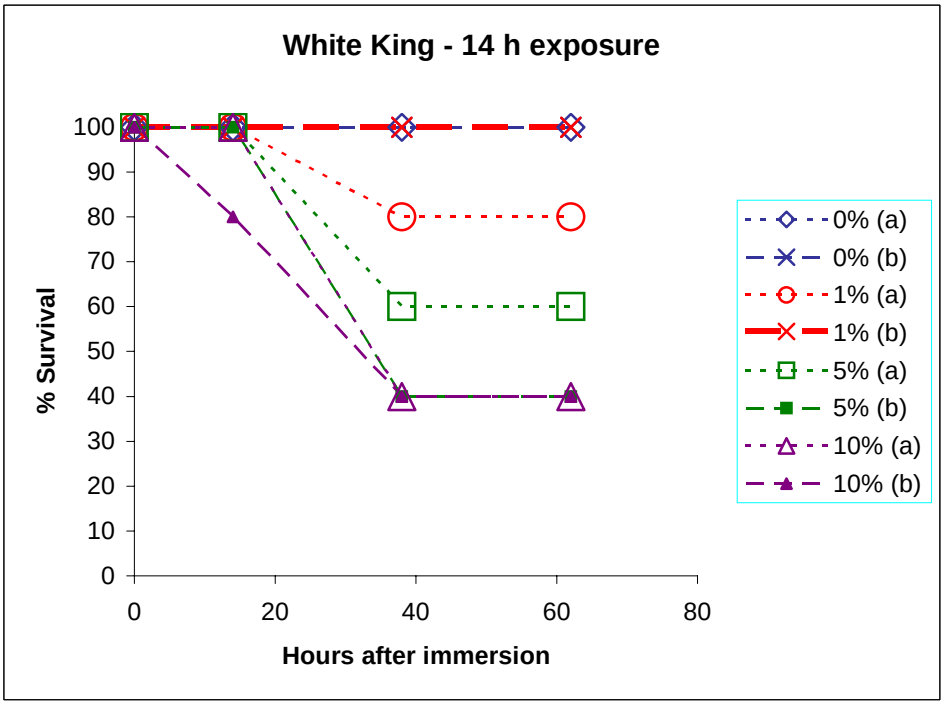

Figure 12. Test results for bleach (14 h exposure)

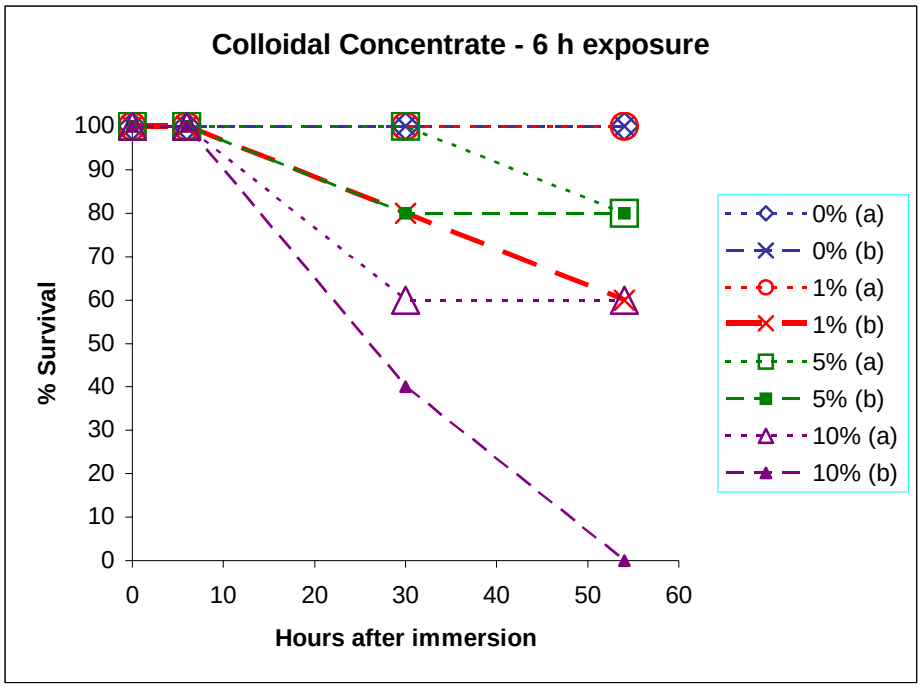

Figure 13. Test results for Pipework Treatment 1 (6 h exposure)

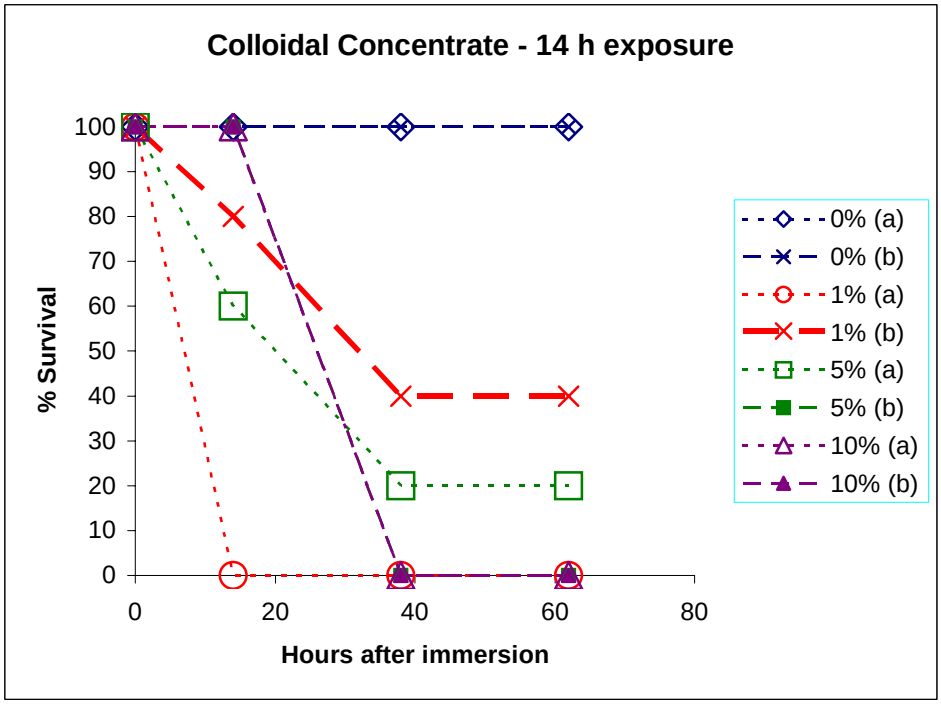

Figure 14. Test results for Pipework Treatment 1 (14 h exposure)

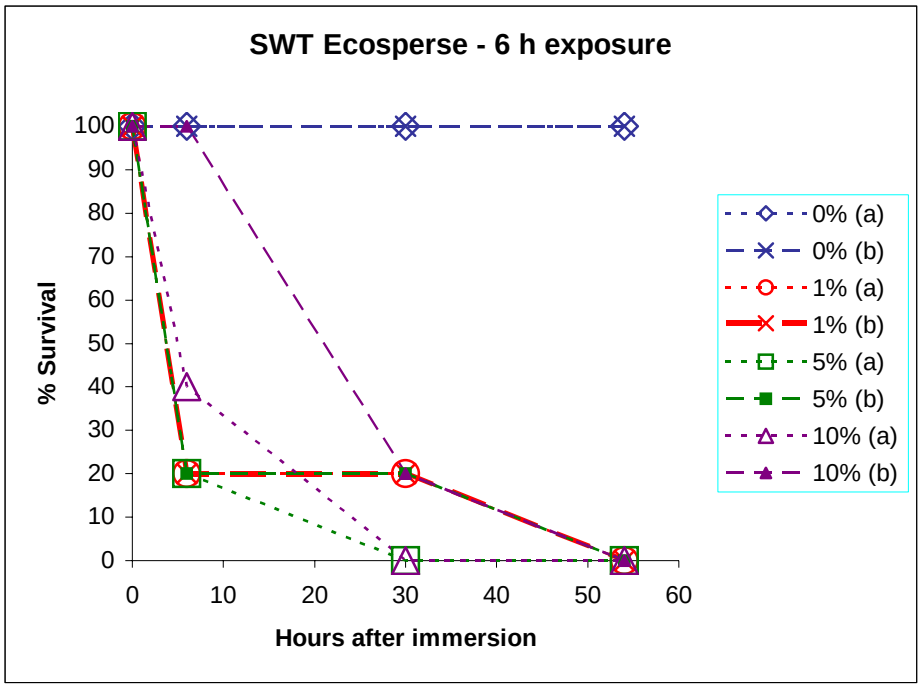

Figure 15. Test results for Pipework Treatment 2 (6 h exposure)

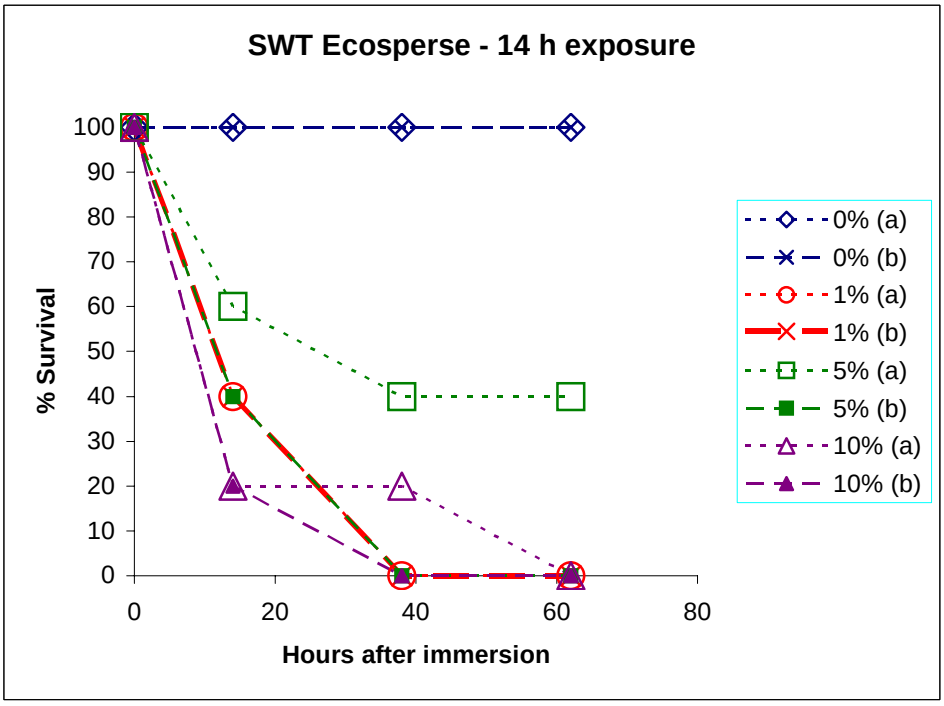

Figure 16. Test results for Pipework Treatment 2 (14 h exposure)

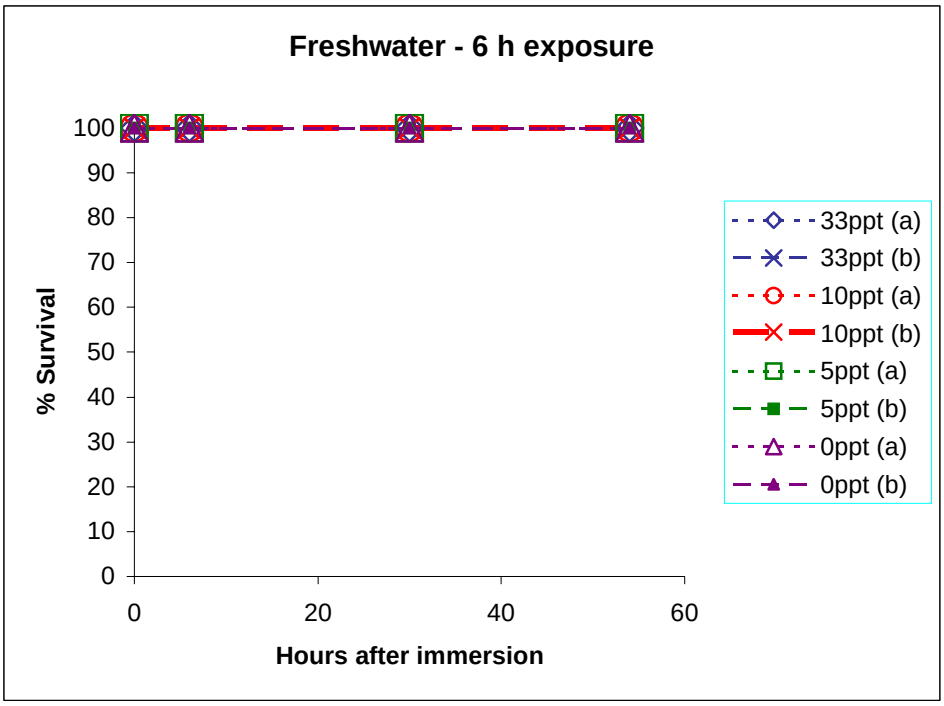

Figure 17. Test results for freshwater (6 h exposure)

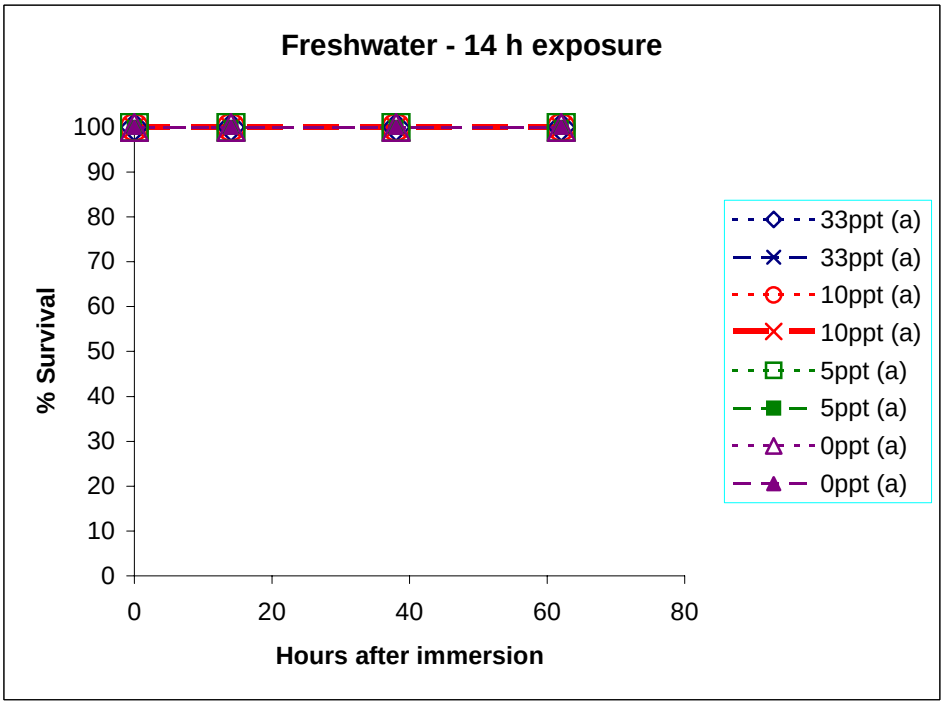

Figure 18. Test results for freshwater (14 h exposure)

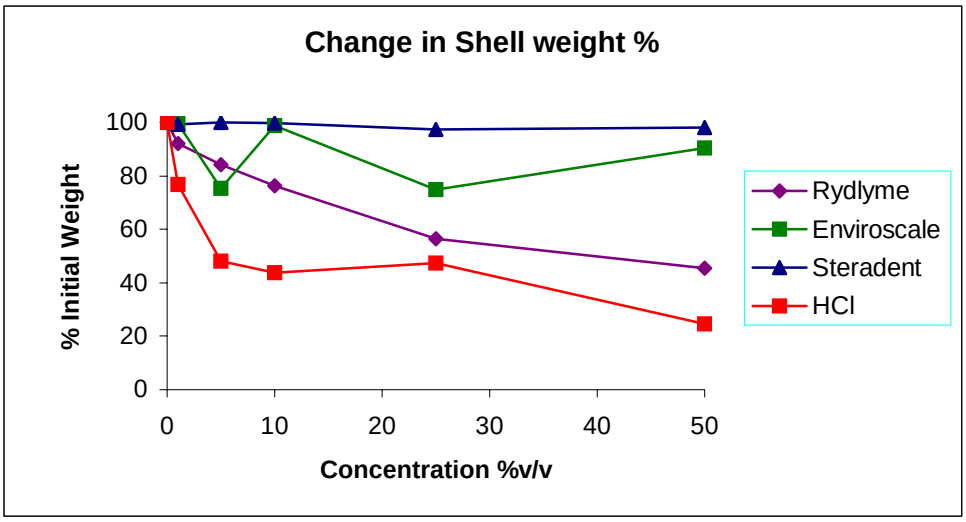

Figure 19. Change in shell weight, as a percentage of initial weight, at different descaler concentrations

Note: Steradent results are for 1, 2, 3, 4 & 5 tablets

5. Discussion

With the exception of freshwater, all of the treatments tested caused some mortality. However, for most, the effect was variable and some mussels did survive 6 or 14 h exposures.

Two treatments caused 100% mortality, at all concentrations tested, for 14 h exposures: the two disinfectants *Conquest* and *Quatsan*. *Quatsan* toxicity appeared higher than *Conquest*, with higher overall mortality in 6 h tests and, in 14 h tests, all mussels clearly dead within 24 h of the test exposure. Material Safety Data Sheets (MSDS) for these two products (see Section 3.1) indicate that *Quatsan* has a significantly higher content of quaternary ammonium compounds than *Conquest*. The MSDS for *Conquest* does not specifically list benzalkonium chloride as an ingredient, only the class of chemicals, "quaternary ammonium compounds", that encompasses this compound. Chemical analysis confirmed the presence of alkylbenzyldimethylammonium chloride in both products, with *Quatsan* determined to contain approximately 30% more of this quaternary ammonium species than the *Conquest* disinfectant (Annex 1). Benzalkonium chloride is a mixture of alkyldimethylbenzylammonium chlorides where the alkyl group may vary from C8H17 to C18H37 (ChemWatch 2002).

Both *Conquest* and *Quatsan* are commercial grade disinfectants containing surfactants, alkaline salts, and quaternary ammonium compounds, including benzalkonium chloride. Benzalkonium chloride is rated to be of moderate toxicity and its use is generally as a germicide and fungicide (ChemWatch 2002). Benzalkonium chloride is also known to have antifouling activity (Parr *et al.* 1996, Jenner *et al.* 1998, Chou *et al.* 1999, Cowie *et al.* 2006).

Following the 1999 BSM incursion in Darwin, the Northern Territory adopted a protocol for the treatment of internal seawater systems for potential marine pests involving the introduction of a 5% (in seawater) "detergent " solution (*Conquest*) into the pipework of the vessel, and closing the seacocks to retain the solution in the system for a minimum period of 14 h. This protocol was based on the findings of the Northern Territory University (NTU) in testing undertaken at the time of the Darwin BSM incursion (D. Parry, pers. comm.). In the NTU experiments, the toxicity of *Conquest TGA* to BSM was evaluated alongside several domestic detergents "off the supermarket shelf". *Conquest TGA*, at 1% v/v in 19 and 33 ppt salinity seawater, gave LT_{100s} of 7 h, compared with LT_{100s} of 24 h for domestic detergent. "LT_{100}" is the time to kill 100% of test organisms. *Conquest TGA*, also manufactured by Shamrock Chemicals (N.T.) Pty Ltd, differs to *Conquest* in being a 'cleaner sanitiser', which is not presently reported to contain quaternary ammonium compounds[13]. Listed chemical constituents include: surfactants <10%, ethyleneglycol monobutyl ether <10 %, alkaline salts <10%, and other unspecified, non-hazardous ingredients <10%.

In North America, the product *Clamtrol CT-1* ® is marketed as a molluscicide for zebra mussel control. The active ingredient in *Clamtrol* is 13% benzalkonium chloride (Waller *et al.* 1993). Waller *et al.* investigated the molluscicidal activity of a range of chemicals against zebra mussels, and found that *Clamtrol*, although not the most toxic of the chemicals tested, showed greater selectivity for zebra mussels than to non-target species of fish and mussels, and was effective at a concentration of 1.0 mg/L.

In contrast, Neil and Stafford (2005) found *Quatsan* at concentrations of 5 and 10% to be ineffective in killing the Sydney rock oyster (*Saccostrea glomerata*) under experimental conditions. For 12 h exposures, less than 20% of oysters exposed to 10% *Quatsan* were killed. This result may be comparable to that of Waller *et al.* (1993) who found higher toxicity of *Clamtrol* to zebra mussels than to a unionid mussel.

Neil and Stafford (2005) also drew attention to the reported relationship between the effectiveness of non-oxidising chemicals, such as quaternary ammonium compounds, and water temperature, with effectiveness typically increasing with increasing temperature (Jenner *et al.* 1998). The experiments in this mussel study were conducted at 20°C and it is therefore possible that the disinfectants may be even more effective in killing mussels in tropical waters, or less effective in colder waters.

The suitability of benzalkonium chloride based treatments for discharge to the environment was assessed by Neil and Stafford (2005) who concluded that a 5% solution of *Quatsan* could be safely released to the environment only by ensuring proper dilution of the solution occurred. Their Microtox analysis of effluent water from 10% *Quatsan* indicated the need for dilution approximately 13000 times for safe release to the open environment. Neill and Stafford (2005) proposed that best practice would be to release waste water to an onshore dilution facility to ensure it was properly diluted prior to release to the environment. In regard to effluent toxicity, it may be significant that, from the chemical composition data listed

[13] Material Safety Data Sheet: Conquest TGA Sanitiser. Issue Date: December 06. Shamrock Chemicals (N.T.) Pty Ltd

on Material Safety Data Sheets (Section 3.1), the quaternary ammonium compound content reported for *Quatsan* is higher than for *Conquest*.

One unexpected observation in the 6 h immersion tests of *Conquest* was that highest mortality was observed at the lowest concentration (1% v/v) with some survival seen in one test at both 5% v/v and 10% v/v. Total mortality occurred in 14 h immersion tests. One hypothesis for this is that at low concentrations mussels do not sense the biocide in the water and continue to actively feed. At higher concentrations the mussel responds to the chemical stimulus and closes its shell. However, unlike the reaction to freshwater (see below) the mussels do not appear able to survive longer exposures.

Both the disinfectants and the detergents generated large amounts of foam during the testing. This was stimulated by the air bubbling used to aerate the test solutions. The degree of foaming is likely to depend on the amount of turbulence or other movement of dosed seawater. If the treatment chemical is dosed and circulated without turbulence, foaming is likely to be reduced. However, the toxicity of the disinfectants under static and turbulent conditions may vary and further testing may be necessary to explore this relationship.

A recommendation to use these disinfectants as biofouling treatments may require assessment and approval by the Australian Pesticides and Veterinary Medicines Authority (APVMA). This needs to be clarified with the APVMA.

Neil and Stafford (2005) found that a 10% vinegar solution exposure for a 12 h period, though not 100% effective, consistently killed over 75% of test oysters. Similar mortality was achieved against *Mytilus* in the present study, with mortalities of between 60 and 100% for both 6 and 14 h exposures. However results were variable, and in one test of 50% vinegar over 14 h, 40% of the mussels survived. Increased replication may reduce the variance in the results, but the important observation remains that vinegar has a variable effect and cannot be guaranteed to cause mortality in 6 or 14 h exposures.

Of the other chemicals tested, *Palmolive* dishwashing detergent, *Triple 7 Colloidal Concentrate,* and *SWT Ecosperse* also caused full mortality in some of the 14 h tests. For *Palmolive*, one test at each concentration (1%, 5%, 10%) caused 100% mortality, but the replicate test did not. 10% *Colloidal Concentrate* killed all mussels in both tests, as did one of the treatments at 1%, but survival did occur in some tests at 1 and 5%. 14 h *SWT Ecosperse* exposures killed all mussels in all but one test at 5%.

The toxic mechanism is likely to vary between these three treatments. D. Parry (pers. comm.) speculated that detergents may kill mussels by denaturation of proteins, and detergents had been noted to have lethal effects in aquaria. Chemical components of *Colloidal Concentrate*, perhaps the tall oil fatty acids, and of *SWT Ecosperse*, neutralised alkyl amine and the unspecified biocide, may have a direct toxic effect. Some fatty amines are known to destroy gill tissues by acting directly on the cell membrane and/or by combination with the mucous layer produced by the gills (Jenner *et al.* 1998).

The two remaining chemical treatments, the detergent *Dobatex* and *White King* bleach, did cause some mortality, but never 100%. Bleach did cause dissolution of byssal threads and, if

an event required mussel dislodgement, this could be applied. However, as mussels did survive this impact, mussels flushed through a system after dislodgement could re-establish after discharge.

Freshwater dilutions and even full immersion in freshwater, caused no mortality in any tests. Mussels generally tolerate a wide range of salinities and a favoured habitat is often brackish or estuarine (Chou *et al.* 1999, Seed and Suchanek 1992). They are also known to be able to acclimate to lowered steady state salinities. With a sufficient decline in salinity, mussels respond by closing their shells and maintaining a relatively high osmotic concentration within the mantle fluid. Feeding is suppressed in this state, so eventually the mussels will starve. Freshwater exposure time for 100% mortality of *Mytilus californianus* was found to be 48 h, and for *Mytilus edulis*, 63 h (Fox and Corcoran 1957).

Descalers are used to remove insoluble deposits from the internal surfaces of pipework, or dentures in the case of *Steradent*. The digestion of mussel by the descalers *Rydlyme* and *Enviroscale* and hydrochloric acid is a chemical degradation of the calcium carbonate shell by the acid. The extent of the reaction is dependent on the availability of acid in the solution. In this study, a weight loss of approximately 50% represents full digestion of the shell, leaving only the soft body parts. 5% of the volume of hydrochloric acid was therefore needed to digest one mussel, and 25% of the volume of *Rydlyme.* Digesting additional mussels would require a linear increase in acid presence proportional to the increase in biomass of the mussels. For example, two mussels in the same volume of test solution would require a 10% concentration of HCl and 50% of *Rydlyme*. Heavily fouled pipework would therefore require high concentrations of acid solution and the practicality and safety of this is questioned.

6. Conclusions

The most effective and reliable chemicals tested against the blue mussel in this test program, conducted at 20°C, were the disinfectants *Conquest* and *Quatsan.* No mussels survived concentrations of 1%, 5% and 10% v/v disinfectant after 14 h exposures. The effect is attributed to both chemicals containing the quaternary ammonium compound benzalkonium chloride, which is known as an antifouling compound and molluscicide. Other chemicals tested, apart from freshwater, also caused some mortality, but not as reliably as for these disinfectants.

The environmental acceptability of release of treatment water containing benzalkonium chloride or other quaternary ammonium compounds warrants further investigation. Also, the need for APVMA approval of disinfectants for the purpose of biofouling control also needs clarification.

7. Acknowledgements

The authors wish to acknowledge Andrew Scardino (DSTO) for assistance in collecting mussels, Helen Cribb (NT Fisheries) for supplying the *Conquest* disinfectant, Gary Mathys (DSTO) for the chemical analysis of disinfectants, and Ashley Coutts (AQIS ACT) for providing constructive comments on the manuscript.

8. References

1. ChemWatch (2002) Benzalkonium chloride. ChemWatch Material Safety Data Sheet (Review). CHEMWATCH 10615. Issue Date: Thu 11-Jul-2002

2. Chou, C.L., Zwicker, B., Porter, J.F. and Pelletier, G.R. (1999) Potential biofouling strategies against blue mussel (*Mytilus edulis*) infestation in a cooling water system. *Canadian Technical Report of Fisheries and Aquatic Sciences* **2283**, vi + 89 pp.

3. Cowie, P.R., Smith, M.J., Hannah, F., Cowling, M.J. and Hodgkeiss, T. (2006) The prevention of microfouling and macrofouling on hydrogels impregnated with either Arquad 2C-75® or benzalkonium chloride. *Biofouling* **22** (3), 173-185

4. Fox, D.L. and Corcoran, E.F. (1957) Thermal and osmotic countermeasures against some typical marine fouling organisms. *Corrosion* **14**, 131t-132t [cited in Chou *et al.* 1999]

5. Jenner, H.A., Whitehouse, J.W., Taylor, C.J.L. and Khalanski, M. (1998) Cooling water management in European power stations: Biology and control of fouling. *Hydroecologie Appliquee* **10** (1-2), 1-225

6. Neil,K.M. and Stafford, H. (2005) Efficacy of treatments against internal biofouling in small vessels. Queensland Department of Primary Industries and Fisheries and CRC Reef, report to the Australian Government Department of Agriculture, Fisheries and Forestry. 30 pp.

7. Parr, A.C.S., Smith, M.J., Cowling, M.J. and Hodgkiess, T. (1996) The antifoulant properties of benzalkonium chloride. *Speciality Chemicals* **16** (5), 157-160

8. Seed, R. and Suchanek, T.H. (1992) Population and community ecology of *Mytilus*. In, Gosling, E. (ed.), *The mussel* Mytilus*: ecology, physiology, genetics and culture*, Amsterdam, Elsevier

9. Waller, D.L., Rach, J.J., Cope, W.G., Marking, L.L., Fisher, S.W. and Dabrowska, H. (1993) Toxicity of candidate molluscicides to zebra mussels (*Dreissena polymorpha*) and selected nontarget organisms. *Journal of Great Lakes Research* **19** (4), 695-702

Annex 1: ANALYSIS OF DISINFECTANTS

Infrared spectroscopic analysis of two samples of disinfectants was requested. These disinfectants were proposed as treatments to kill mussels in shipboard pipework. The Quatsan disinfectant was found to be the most effective disinfectant. Analysis was requested to compare the level of the active species present (quaternary ammonium compound).

The samples submitted were:
Quatsan, Clear solution
Conquest, Magenta coloured solution

Results:

The samples were examined using infrared spectroscopy with a Nicolet 5700 Fourier Transform Infrared Interferometer (FTIR) and its Smart Orbit Diamond Attenuated Total Reflection (ATR) accessory. The disinfectants were identified from their infrared spectrum by comparing them to a reference collection. The samples were also separated using solvent extraction to attempt to identify their different constituents.

Examination of the MSDS for the Quatsan disinfectant indicated that there are two different quaternary ammonium compounds present in the formulation. However, the composition of both quaternary ammonium compounds is almost identical with only the alkyl chain varying slightly in length. This information was extracted using the CAS Registry Number. It indicated that the ingredient listed as Quaternary Ammonium Compound (CAS No. 63449-41-2) and as present in the highest concentration was alkylbenzyldimethyammonium chloride with the alkyl group having a chain length of 10-16 carbon atoms. The ingredient itemised as Benzalkonium Chloride (CAS No. 68989-00-4) has the same chemical structure except the alkyl chain contains 8-18 carbon atoms.

Conquest is specified as containing Quaternary Ammonium Compounds (CAS No. 68624-85-1). The CAS number listed is obviously incorrect as it states that it is a totally different compound (Molecular formula: $C_{29}H_{47}NO_6$, HP=Prost-13-en-1-oic acid (9CI); SB=15-(acetyloxy)-20-cyano-9-((tetrahydro-2H-pyran-2-yl)oxy)-; NM=methyl ester; ST=(13E)-(.PM.)-). The infrared spectrum of the dried disinfectant was consistent with containing an alkylbenzyldimethylammonium chloride.

Since, both disinfectants contain alkylbenzyldimethylammonium chloride species, the relative amounts of this constituent can be determined by comparing the intensity of the mono substitution peak from the benzyl groups at 700 cm^{-1} (out of plane C-H deformation vibration) for a fixed sample thickness. The Diamond Attenuated Total Reflection (ATR) accessory is suitable for the comparison of these aqueous solutions without pre-treatment because it is inert to water and automatically measures a fixed sample thickness. The weak, sharp, peaks of interest appear on top of the broad O-H wagging vibration from the water, but can be

extracted relatively easily by either flattening out the broad water peak or differentiating the spectra. These results suggest that Quatsan contains approximately 30% more of the quaternary ammonium species than the Conquest disinfectant.

The disinfectants were separated using solvent extraction to approximately determine their composition. These results indicated that Quatsan and Conquest only contain 18 and 9% solids (by weight after drying), respectively. Since, both of these disinfectants contain several other ingredients (see Table 1), the concentration of the quaternary ammonium salt is probably less than 5%.

Table 1

Disinfectant	Composition
Quatsan	82% Water Ethoxylated alkylphenol surfactant Alkyldimethylbenzyl ammonium chloride Citrate salt (possibly sodium) Possibly other inorganic species
Conquest	91% Water Polyethylene oxide derivative Alkyldimethylbenzyl ammonium chloride Metasilicate derivative (possibly sodium)

Gary Mathys
Spectroscopy & Thermal Analysis
MPD, PSL

16th January, 2007

DEFENCE SCIENCE AND TECHNOLOGY ORGANISATION
DOCUMENT CONTROL DATA

1. PRIVACY MARKING/CAVEAT (OF DOCUMENT)

2. TITLE

Treatment of Biofouling in Internal Seawater Systems - Phase 2

3. SECURITY CLASSIFICATION (FOR UNCLASSIFIED REPORTS THAT ARE LIMITED RELEASE USE (L) NEXT TO DOCUMENT CLASSIFICATION)

Document	(U)
Title	(U)
Abstract	(U)

4. AUTHOR(S)

John A. Lewis and Jim Dimas

5. CORPORATE AUTHOR

DSTO Defence Science and Technology Organisation
506 Lorimer St
Fishermans Bend Victoria 3207 Australia

6a. DSTO NUMBER	6b. AR NUMBER	6c. TYPE OF REPORT	7. DOCUMENT DATE
DSTO-TR-2081	AR-014-067	Technical Report	November 2007

8. FILE NUMBER	9. TASK NUMBER	10. TASK SPONSOR	11. NO. OF PAGES	12. NO. OF REFERENCES
2007/1047328/1	COM 06/025	DAFF	24	9

13. URL on the World Wide Web

http://www.dsto.defence.gov.au/corporate/reports/DSTO-TR-2081.pdf

14. RELEASE AUTHORITY

Chief, Maritime Platforms Division

15. SECONDARY RELEASE STATEMENT OF THIS DOCUMENT

Approved for public release

OVERSEAS ENQUIRIES OUTSIDE STATED LIMITATIONS SHOULD BE REFERRED THROUGH DOCUMENT EXCHANGE, PO BOX 1500, EDINBURGH, SA 5111

16. DELIBERATE ANNOUNCEMENT

No Limitations

17. CITATION IN OTHER DOCUMENTS Yes

18. DSTO RESEARCH LIBRARY THESAURUS

Biofouling, Mussels, Descaling, Toxicity, Environmental Protection, Marine Fouling Organisms, Marine

19. ABSTRACT

Biofouling in the internal seawater systems of vessels is considered to pose a high risk for the introduction and/or translocation of marine pests and, as part of Australia's new National System for the Prevention and Management of Marine Pest Incursions, options for treating such biofouling are needed. Mussels are of particular concern. In this study, a range of chemicals, including vinegar, detergents, disinfectants, bleach, descalers, pipework treatments and freshwater, were tested on the southern Australian blue mussel, *Mytilus galloprovincialis planulatus*. The effectiveness of descalers in digesting mussel shells was assessed, and the toxicity of other treatments determined in 6 and 14 h exposures. The most effective treatments were two disinfectants, which both contained the active benzalkonium chloride. 14 h treatments with disinfectants of this type were concluded to be the most effective means of killing mussels. However, the toxicity and environmental acceptability of these chemicals warrant investigation in regard to discharge and disposal of treatment solutions.